Modeling Variability and Uncertainty in Risk Assessment:

a Case Study of *Salmonella* in Low a_w Foods and its Use in Decision Making

Organized by: Microbial Modelling and Risk Analysis PDG

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International Association for Food Protection®

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  Chair of MMRA PDG (2014-2016)

Facilitated Discussion

- Questions should be submitted via the Text Chat section at the bottom of the screen.
- Q&A's to be held at the end of presentation

Modeling Variability and Uncertainty in Risk Assessment:

a Case Study of *Salmonella* in Low a_w Foods and its Use in Decision Making

Sofia Santillana Farakos
Régis Pouillot
Jenny Scott

IAFP WEBINAR June 8th 2016

Before we start…

The information and conclusions presented in this webinar do not necessarily represent new Agency policy nor do they imply an imminent change in existing policy

Outline of today's webinar

- Introduction
- *Salmonella* in low water activity foods
  - Prevalence and levels of contamination
  - Survival
  - Predictive modelling
- Modelling uncertainty and variability when assessing risk
  - The inference process
  - Simulation
  - Challenges
- Variability and uncertainty in risk management
  - Usefulness when making decisions

Variability and Uncertainty

Variability

Heterogeneity

- Not reduced by additional data
- May be better characterized
- Growth, inactivation, serving size, …

Uncertainty

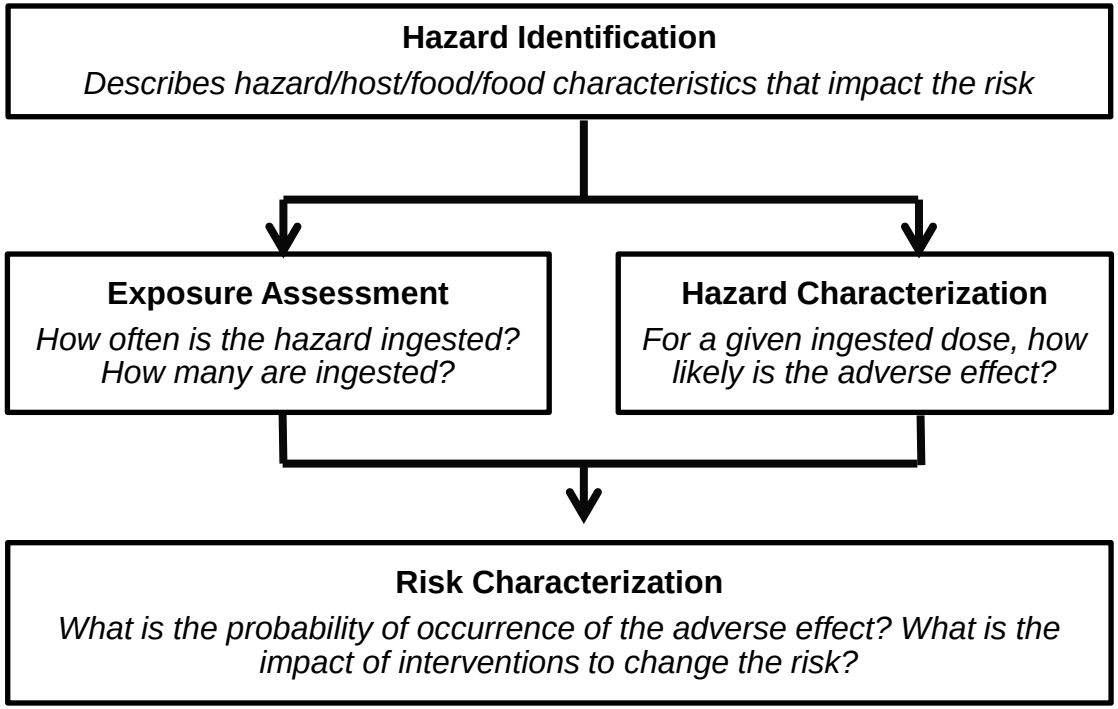
The unknown

- Reduced by additional data
- Dose response, storage times, serving size, …

In many cases, factors are both variable and uncertain

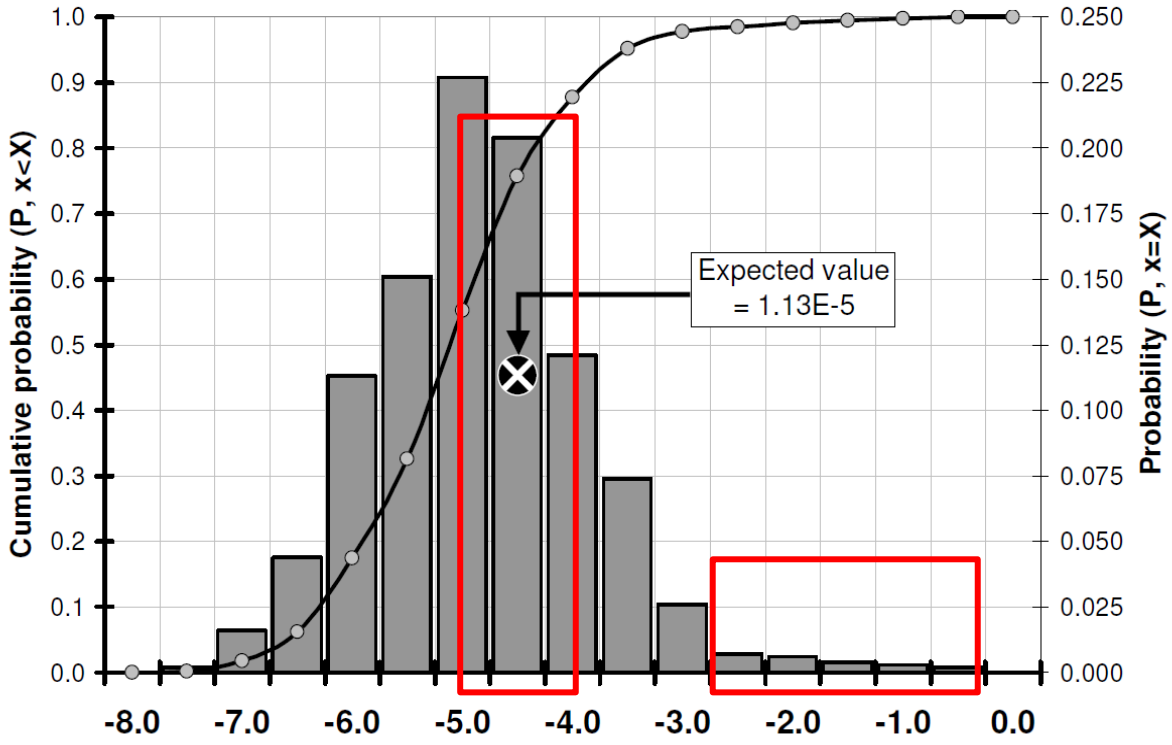
Quantitative Microbial Risk Assessment

Assess the risk of illness from consumption of a product by a population



- Initial contamination
- Initial concentration
- Process characteristics
- Storage conditions
- Serving size
- Dose
- Dose Response
- Etc.

Quantitative Microbial Risk Assessment: An example



Log Risk Per Serving

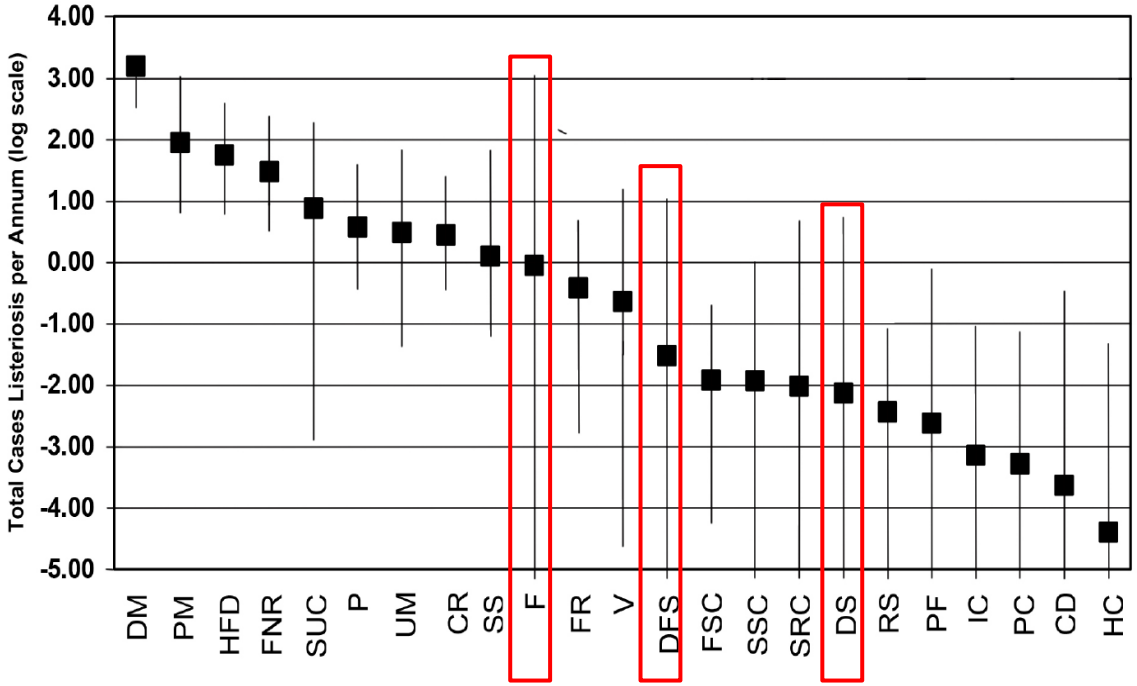
Understanding extremes

CAN HELP RISK MANAGEMENT

FAO/WHO (2002). Risk Assessment of *Salmonella* in eggs and broiler chickens. Technical report. Microbiological Risk Assessment Series, no 2. Rome, Food and Agriculture Organization of the United Nations and World Health Organization: 327

Variability and Uncertainty in Risk Assessment

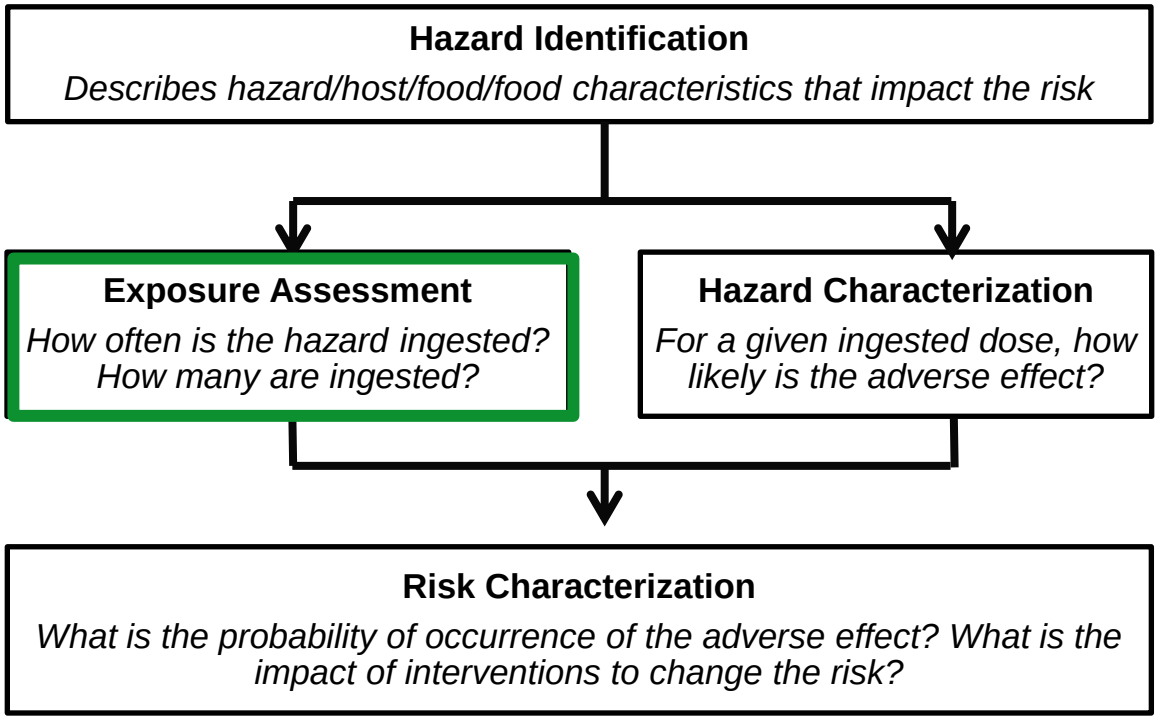
FDA/FSIS (2003). Quantitative assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm183966.htm



DM = Deli meats; PM = Pasteurized Fluid Milk; HFD = High Fat and Other Dairy Products; FNR = Frankfurters (not reheated); SUC = Soft Unripened Cheese; P= Pâté and Meat Spreads; CR = Cooked Ready-To-Eat Crustaceans; UM= Unpasteurized Fluid Milk; SS= Smoked Seafood; F = Fruits; FR = Frankfurters (reheated); V = Vegetables; DFS= Dry/Semi-dry Fermented Sausages; FSC = Fresh Soft Cheese; SSC = Semi-soft Cheese; SRC = Soft Ripened Cheese; DS = Deli-type Salads; RS = Raw Seafood; PF = Preserved Fish; IC= Ice Cream and Frozen Dairy Products; PC = Processed Cheese; CD = Cultured Milk Products; HC = Hard Cheese.

Salmonella in low a_w foods

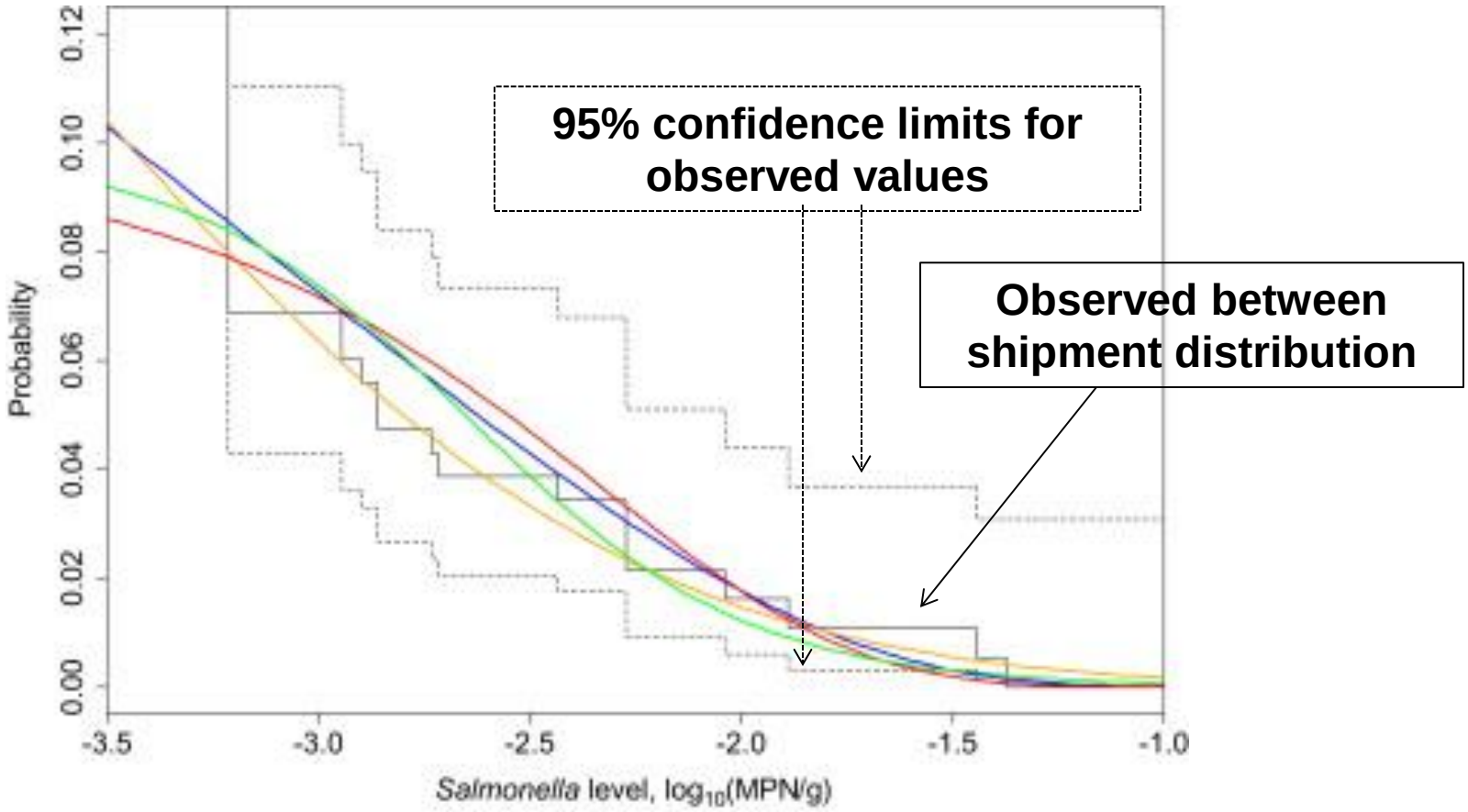
Salmonella in low a_w foods: Contamination level



- Prevalence
- **Contamination level**
- Survival
- Growth
- Dose-Response
- Consumption

- **Variability and uncertainty**
- **From year to year, from lot to lot, intra lot**

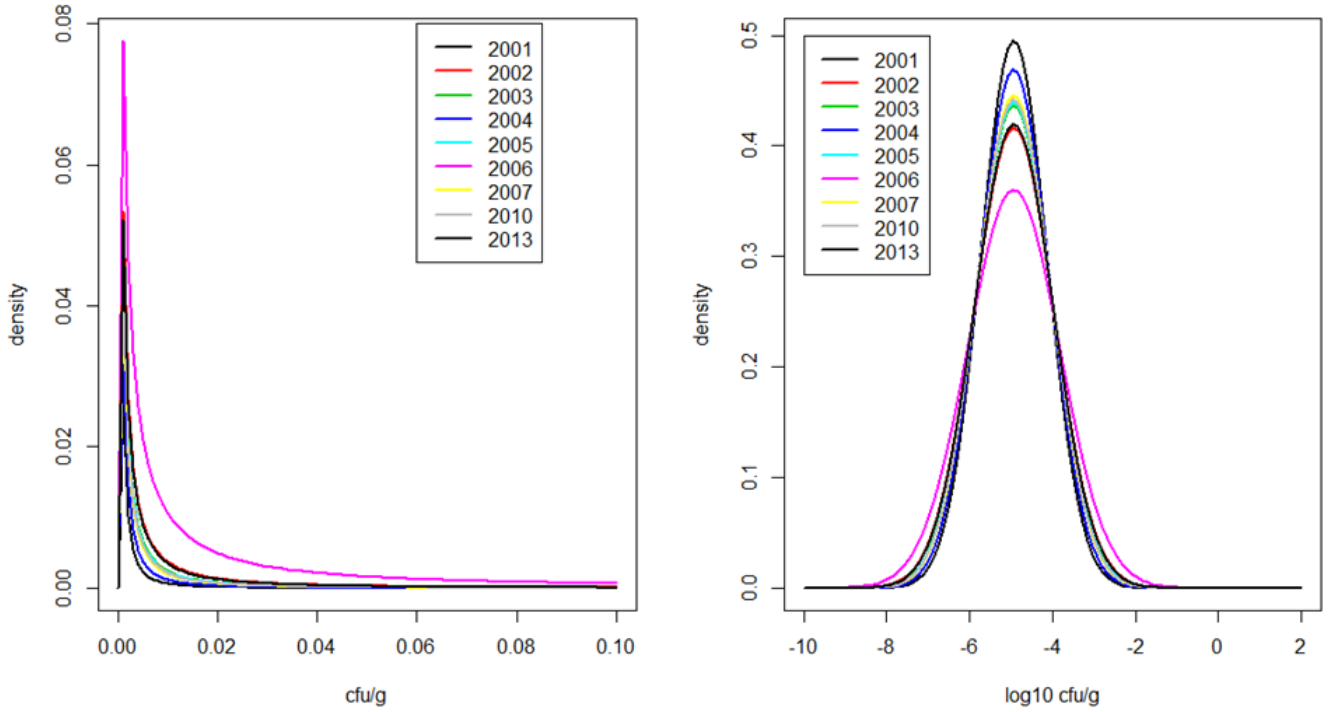
Salmonella in sesame seeds; Variability



Van Doren, J.M., Blodgett, R.J., Pouillot, R., Westerman, A., Kleinmeier, D., Ziobro, G.C., Ma, Y., Hammack, T.S., Gill, V., Muckenfuss, M.F., Fabbri, L., 2013. Prevalence, level and distribution of Salmonella in shipments of imported capsicum and sesame seed spice offered for entry to the United States: Observations and modeling results. Food Microbiology 36, 149-160.

Salmonella in almonds; Variability

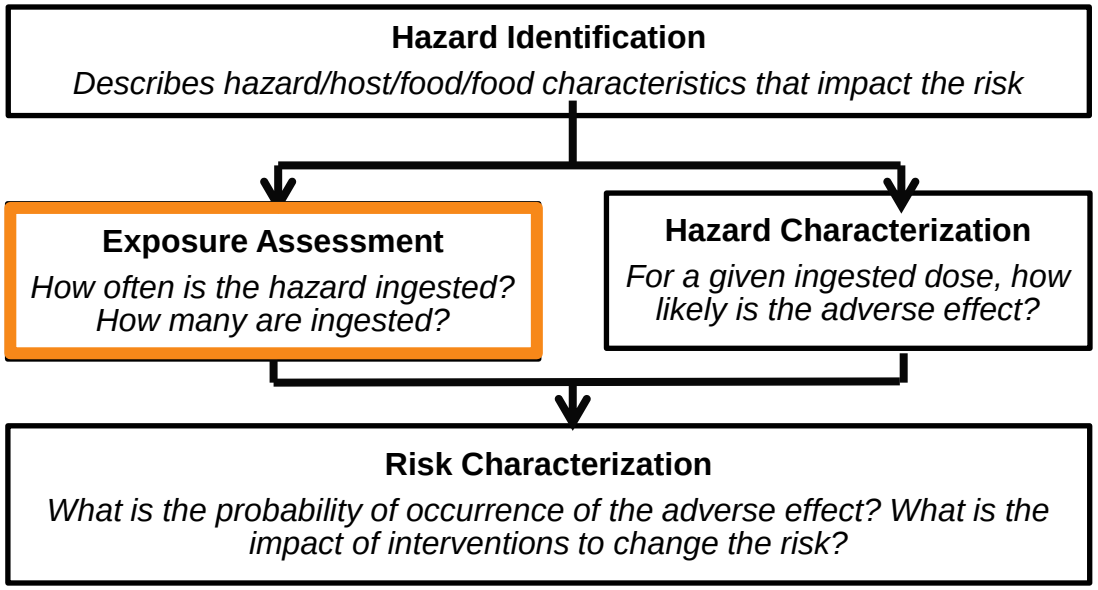
Intra Lot: Poisson distribution
Inter Lot: Log normal distribution
Year to year: same mean of the log, varying standard deviation



Probability density functions of *Salmonella* contamination as predicted by the model.

Data: Unpublished data submitted to Federal Docket through Federal Register Notice FDA-2013_N-0747
https://www.federalregister.gov/articles/2013/07/18/2013-17211/assessment-of-the-risk-of-human-salmonellosis-associated-with-the-consumption-of-tree-nuts-request

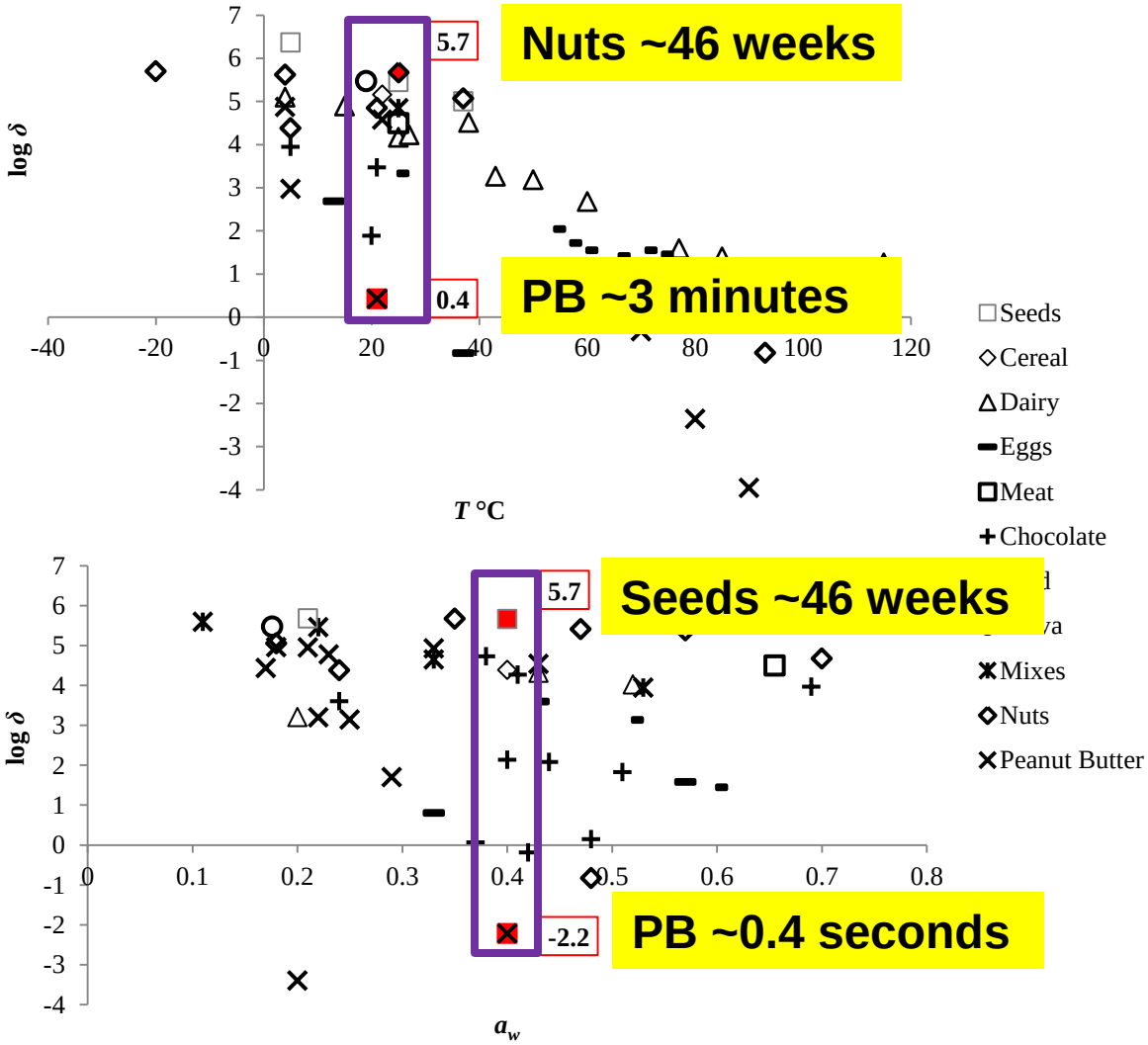
Salmonella in low a_w foods- Survival Data



- Prevalence
- Contamination level
- **Survival**
- Growth
- Dose-Response
- Consumption

Variability and Uncertainty in survival:

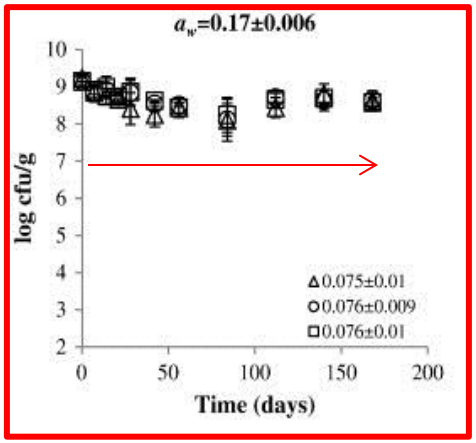
- **Experimental conditions**
- **Strain**
- **Temperature**
- **a_w**
- **Food composition**
- **Survival model parameters**

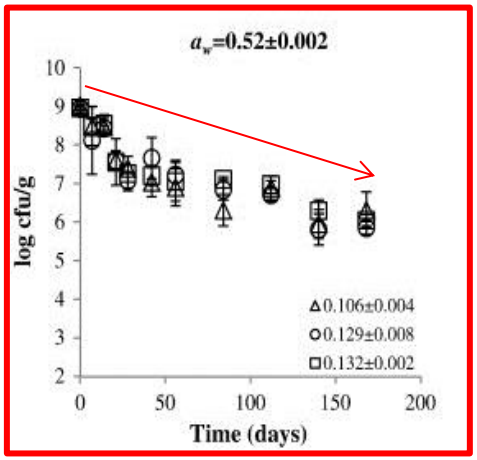


Santillana Farakos, S.M., Schaffner, D.W., Frank, J.F., 2014. Predicting Survival of *Salmonella in Low Water Activity Foods: An Analysis of Literature Data. J Food Prot 77, 1448-1461.*

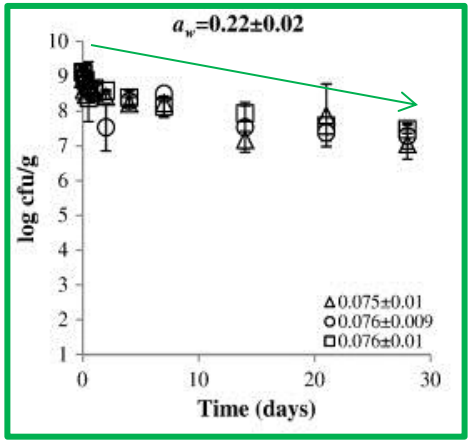
Salmonella spp. survival at various T and a_w on whey protein powder

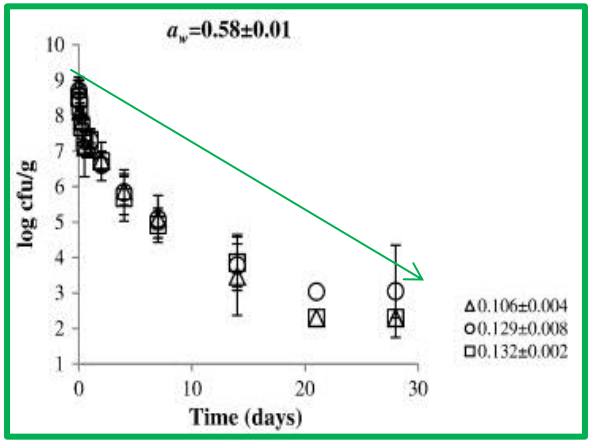
21 °C



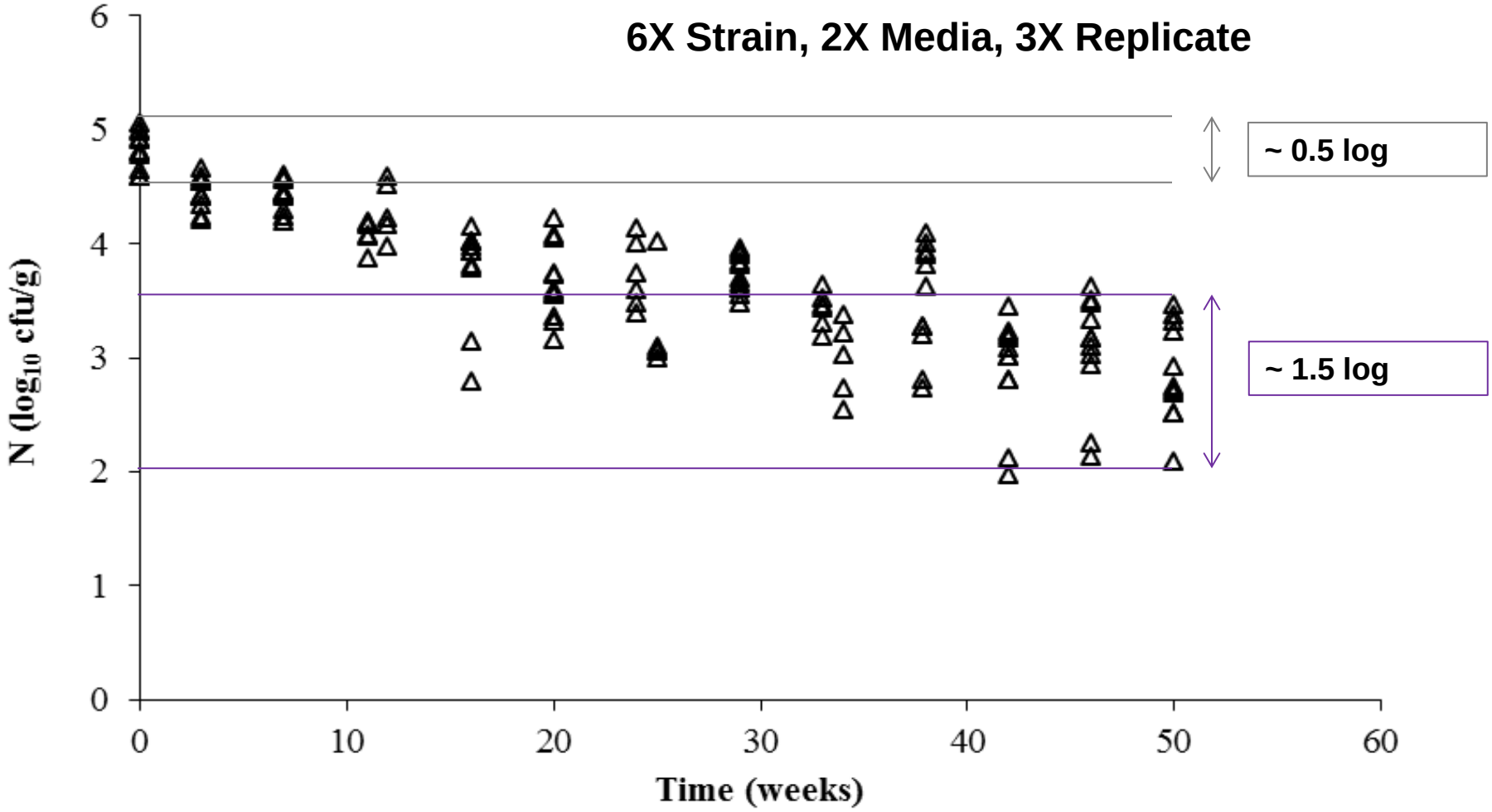


50 °C





Santillana Farakos, S.M., Frank, J.F., Schaffner, D.W., 2013. Modeling the influence of temperature, water activity and water mobility on the persistence of Salmonella in low-moisture foods. Int J Food Microbiol 166, 280-293.



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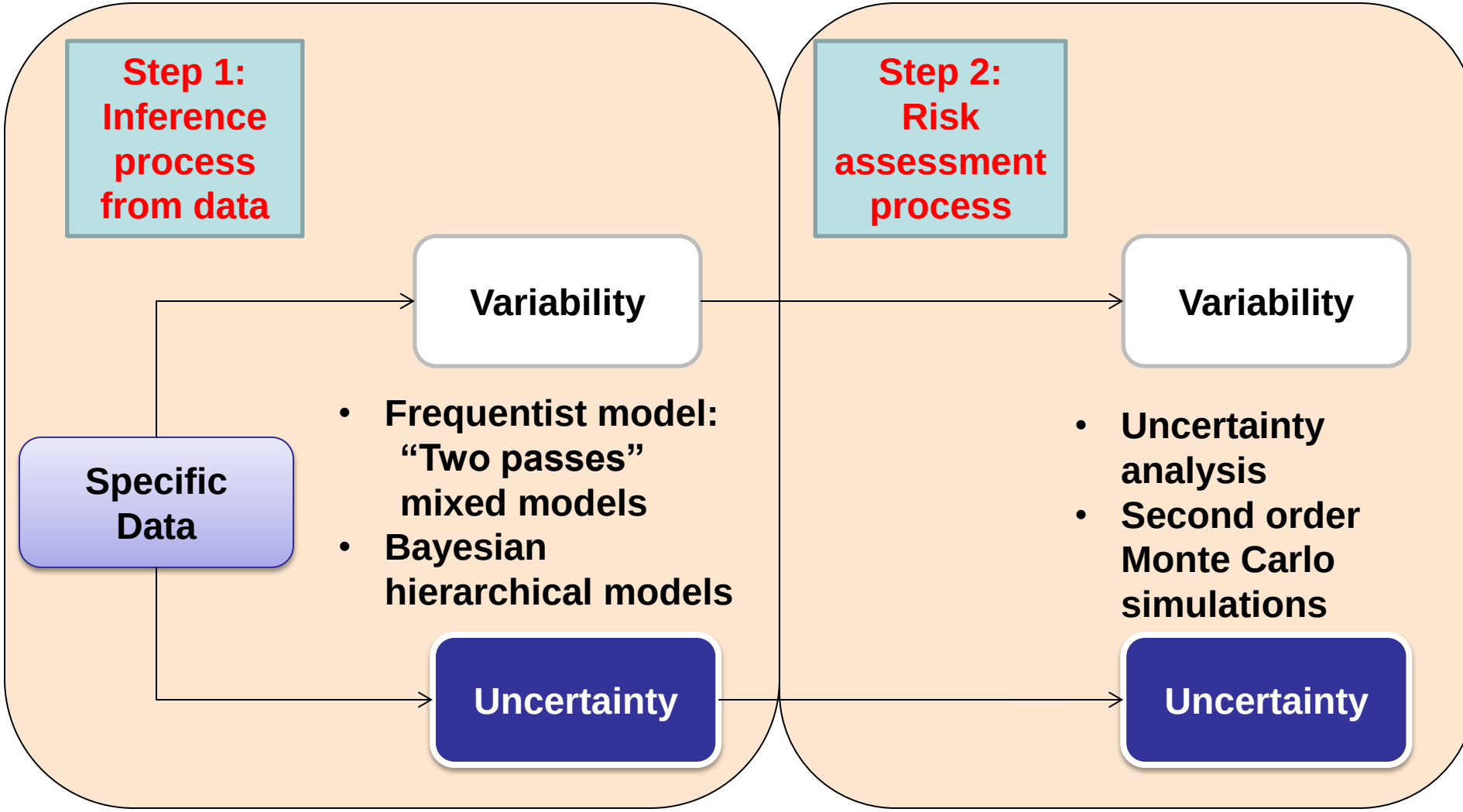
How do we model this variability and uncertainty for use in risk assessment?



(One pass: Bayesian model) (see Albert et al., 2008)

Inference: Frequentist

- Two passes (example: inactivation data)
  - Obtain data from representative situations (strain, stress, …)
  - Model individual datasets for each strains/conditions → get a set of parameters
  - Parameter variability distribution derived from this set of model parameters

Table 2
Decline of *Salmonella* on almond kernels at different temperatures, modeled with linear and exponential functions.

Temperature (°C)	Serotype	Trial duration (days)	Inoculum (log CFU/g)	Linear model[a]	
				Slope (log CFU/month)	p-value[c]
23	PT 30	171	7.1	−0.32	<0.001
	PT 30	336	8.5	−0.21	<0.001
	PT 30	559	7.9	−0.24	<0.001
	PT 30	161	7.3	−0.16	<0.001
	PT 30	161	4.8	−0.21	<0.001
	PT 30	161	3.1	−0.25	<0.001
	PT 30	161	1.2[d]	−0.20	<0.001
	Cocktail[e]	172	5.8	−0.29	<0.001
4	PT 30	171	7.2	−0.052	0.0030
	PT 30	336	8.5	−0.039	<0.001
	PT 30	559	7.9	−0.018	<0.001
	Cocktail[e]	172	5.8	−0.019	0.67
−20	PT 30	559	7.9	0.0027	0.45
	Cocktail[e]	172	5.8	−0.043	<0.001

Slope ~Normal(−0.0078388, 0.00178)

[a] The linear model was expressed as: log(concentration) = log (initial concentration) + rate·days.
[b] The fitted exponential curve was expressed as: log(concentration) = plateau + span·exp(− k·days).
[c] The 95% confidence intervals never included zero, except for the last trial at 4 °C, and the first trial at −20 °C.
[d] The results of this trial were excluded from the calculation of the average reduction rate at 23 °C for risk assessment purposes, due to several non-detects.
[e] The *Salmonella* cocktail included the following serotypes: Enteritidis PT 30, Enteritidis PT 9c, Tennessee, Oranienburg, Anatum, and Montevideo (unpublished).

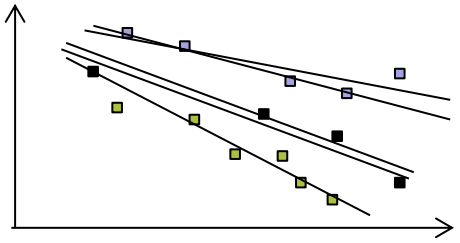
Inference: Frequentist

- One pass, directly from data
  - Mixed (linear or non linear) models are ways to consider population variability.

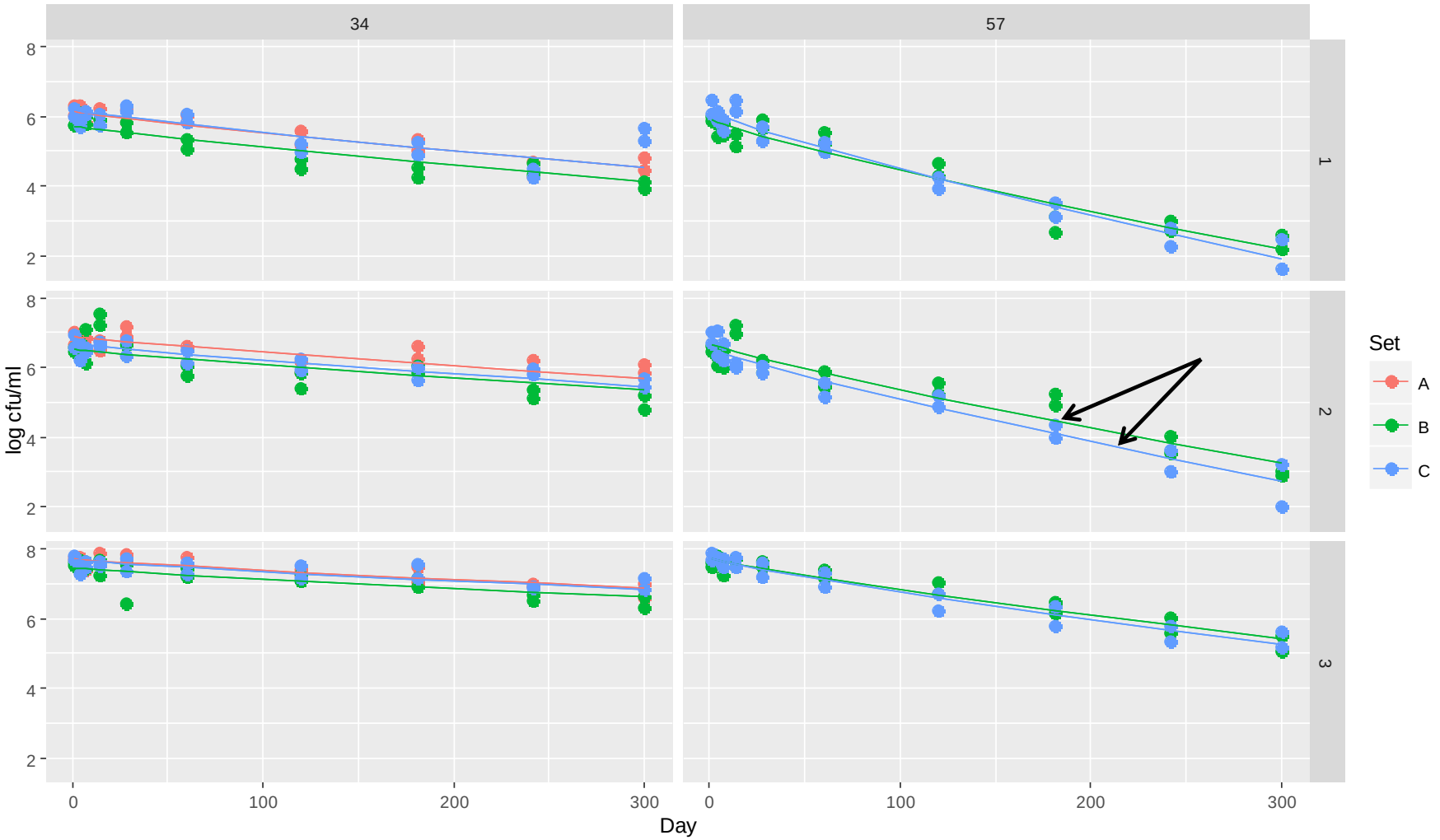
  - Example: (log-)linear inactivation curves obtained from a set of strains *i*

$$y_{ij} = \alpha_{ij} + \gamma_i x + \varepsilon_{ij},$$
$$\gamma_i \sim \text{Normal}(\beta, \sigma_1^2)$$
$$\varepsilon \sim \text{Normal}(0, \sigma_2^2)$$

(rather than $y_{ij} = \alpha_{ij} + \beta x + \varepsilon_{ij}$),



  - The (log-)decrease varies from one strain to the other

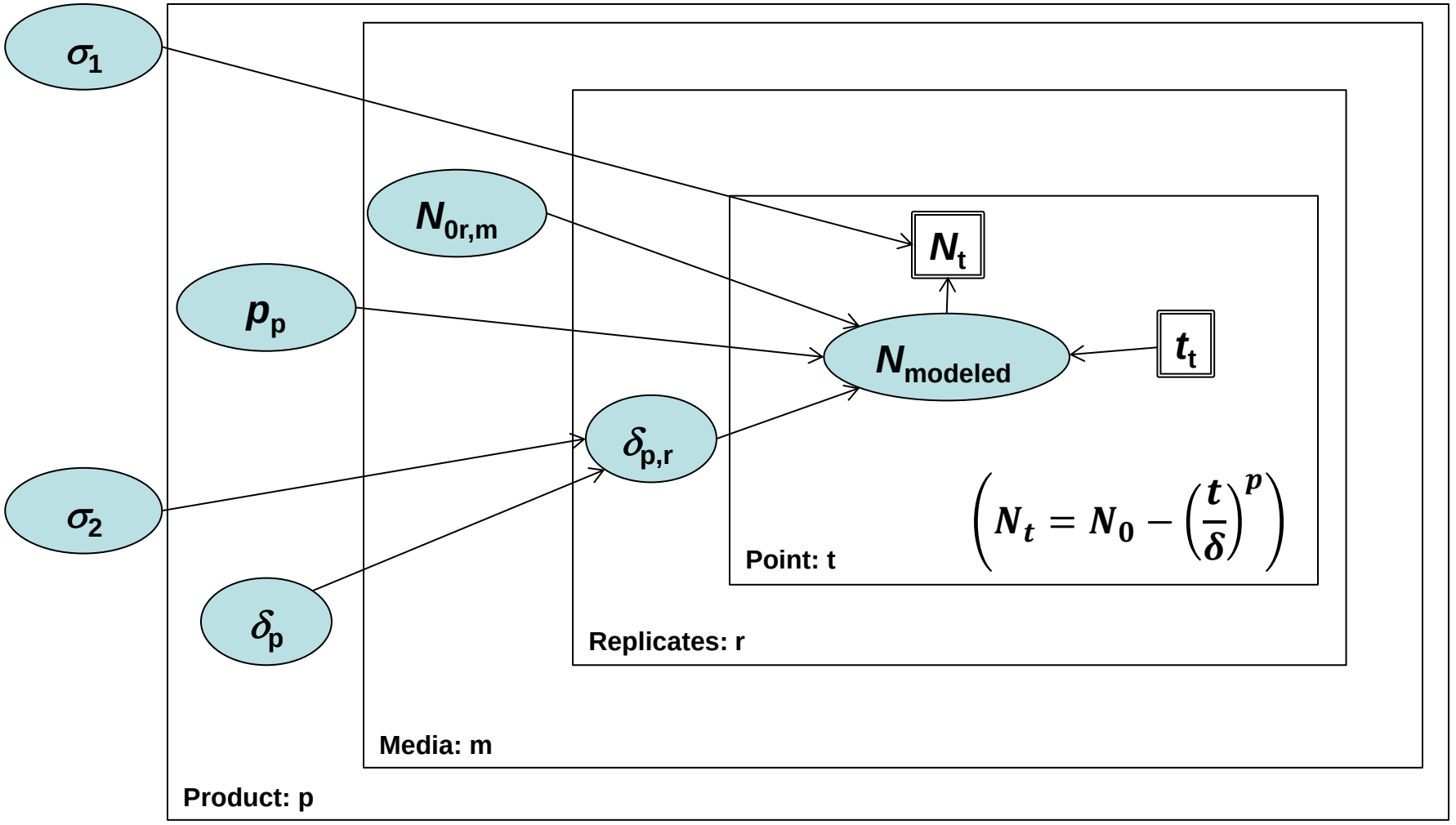


Variability from replicate to replicate $\delta_i \sim \text{Normal}(171, 4^2)$

Inference: Frequentist

- Uncertainty
  - Asymptotic distribution of estimators
    - Usually: normal
    - Difficulty to consider correlations
    - (sample size?)
  - Bootstrap
    - Pros: set of parameters that can be incorporated in the risk assessment model

Inference: Bayesian



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Issue: Ad-hoc experimental design

- Variability
  - From population to population (strain-to-strain)
  - From day-to-day
  - From cell-to-cell within a population
- Need specific experimental design
  - No “cocktail” of strains, no “average” over replicates
  - Control of strain-to-strain, day-to-day conditions
  - See, e.g. den Besten et al., 2016
    - Experimental < Reproduction < Strain = Growth History = Population for *L. monocytogenes* inactivation

- Ad-Hoc data
  - Specific experimental design

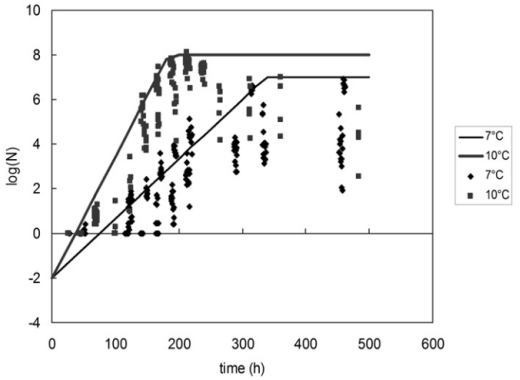


Fig. 3. Variability in the level of *Bacillus cereus* in several cartons of pasteurised milk stored at 7 and 10 °C (adapted from Zwietering et al., 1996).

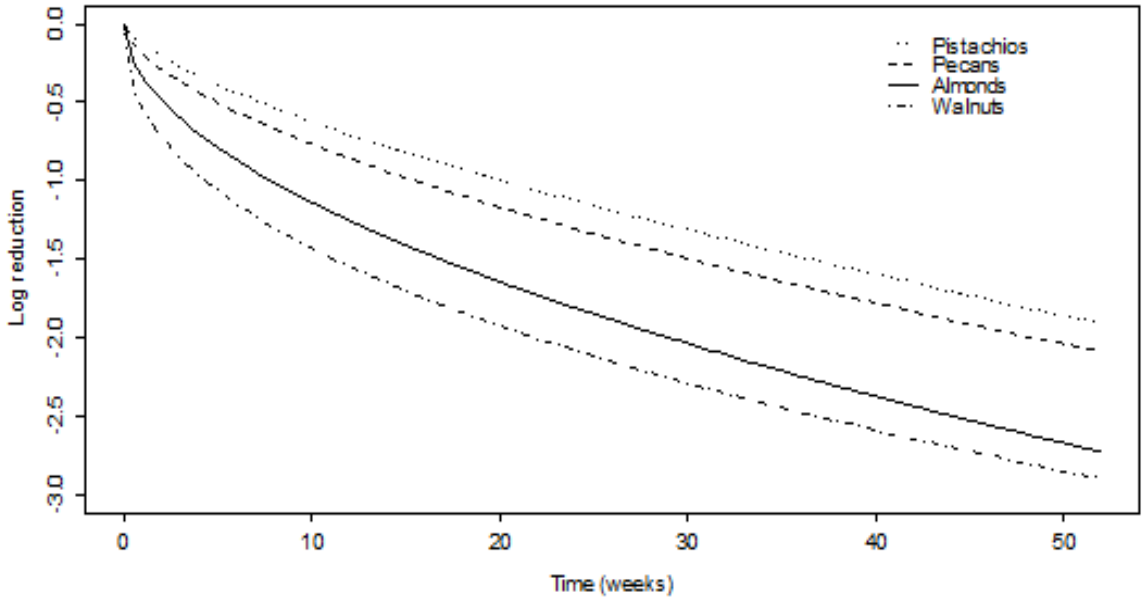
- Literature data (meta analysis)
  - Representative strains?
  - How to consider lab-to-lab variability?

Example: *Salmonella* survival on tree nuts

Study reference	Almond	Pecan	Pistachio	Walnut
Abd et al. (2012)	6	-	-	-
Beuchat and Mann (2010)	-	3	-	-
Blessington, Theofel and Harris (2013)	24	-	-	18
Blessington, Theofel, Mitcham, et al. (2013)	-	-	-	6
Brar et al. (2015)	-	4	-	-
Kimber et al. (2012)	6	-	6	-
Uesugi et al. (2006)	38	-	-	-

$$\log_{10}(N_t) = \log_{10}(N_{0,e}) - (t/(\delta_s + r_\delta))^{\rho_s} + \varepsilon \qquad (3)$$

where N_0, N_t, t, δ, ρ, e, and ε are defined as above with s in δ_s and p_s representing the tree nut, almonds, pecans, pistachios or walnuts, and with the random effect on r_δ following $Normal(0, \sigma_\delta)$.



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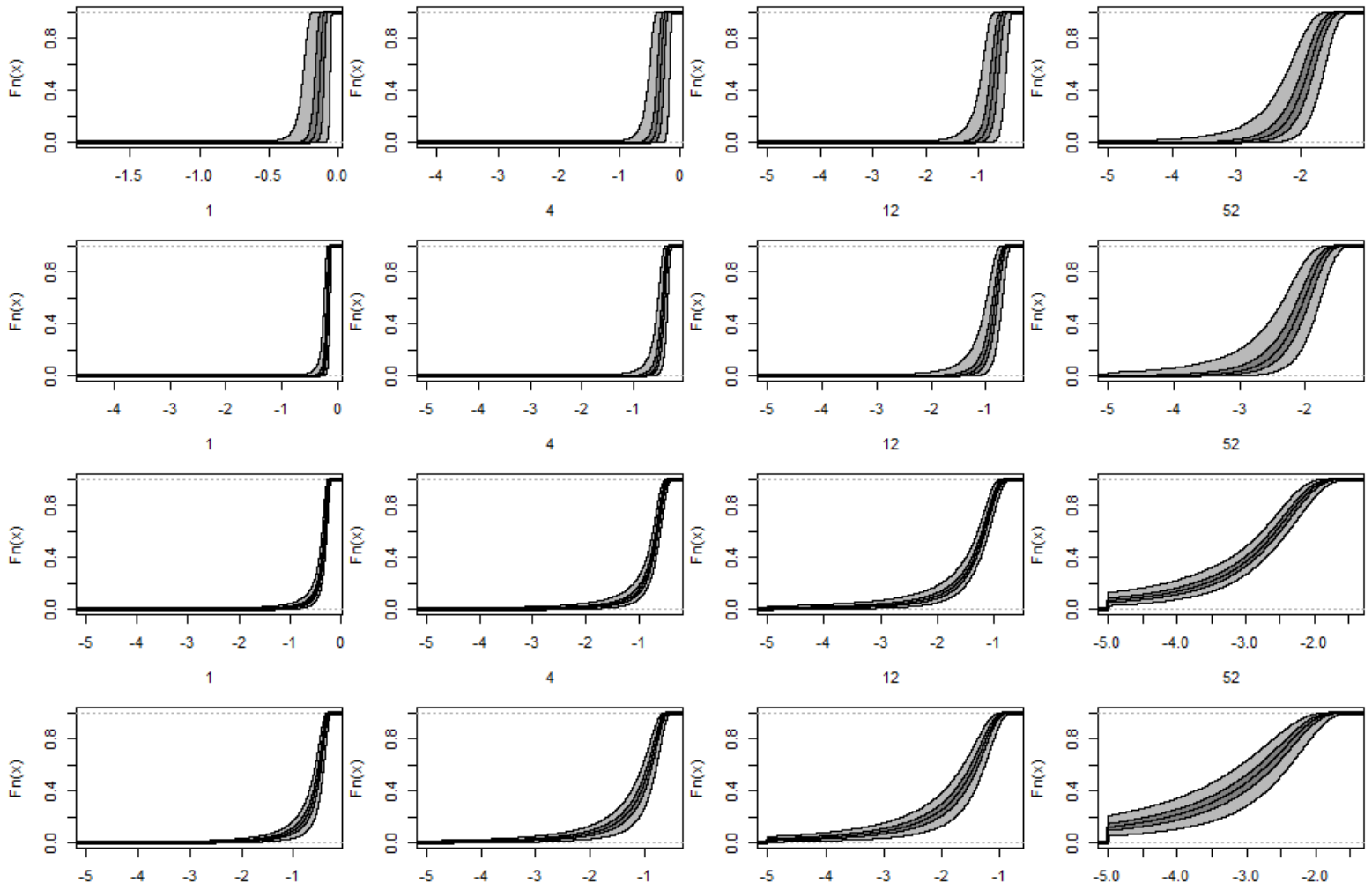

Log Reduction

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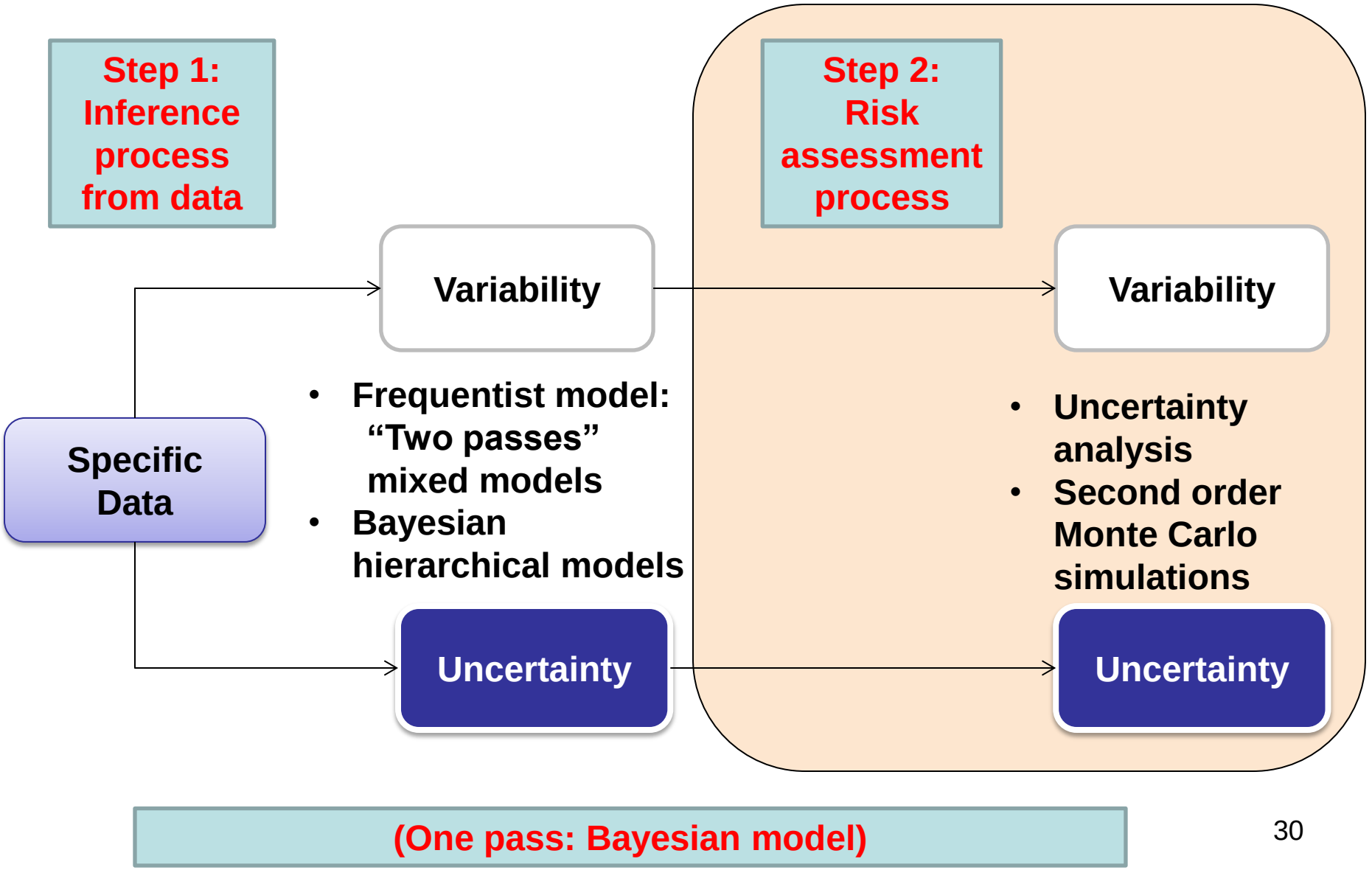
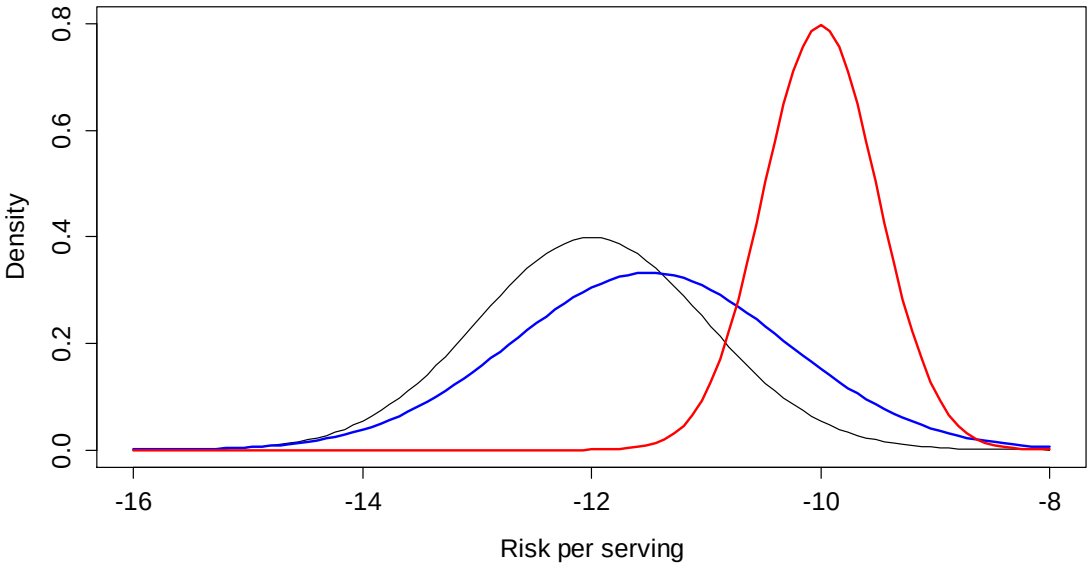

Step 1: Inference process from data
Step 2: Risk assessment process
Variability
Variability
Specific Data
• Frequentist model: "Two passes" mixed models
• Bayesian hierarchical models
• Uncertainty analysis
• Second order Monte Carlo simulations
Uncertainty
Uncertainty
(One pass: Bayesian model)

Test alternative values for uncertain parameters / model

- Baseline
- Alternative #1: little impact
- Alternative #2: high impact



Two-Dimensional (or Second Order) Monte-Carlo simulation

- Principle
  - Separation of the parameters according to the meaning of their dispersion
    - Variable parameters
      - Example: Portion Size (from individual to individual), Distribution of the contamination| mean contamination (from year to year)
    - Uncertain parameters
      - Example: Mean of the number of bacteria / 100g for a given year
  - Model Integration using Two Embedded Monte-Carlo Simulations
    - A Variability modeling embedded in an Uncertainty modeling

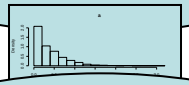
Second-Order Monte Carlo Simulation

Uncertain parameter *X* (example: mean $\log_{10}$ decrease / week)
Fixed to a given random sample issued from the uncertainty distribution

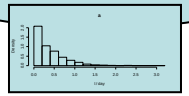
Uncertain parameter *X* (example: mean $\log_{10}$ decrease / week)
Fixed to a given random sample issued from the uncertainty distribution

…

QRA Model (variable parameters)



QRA Model (variable parameters)



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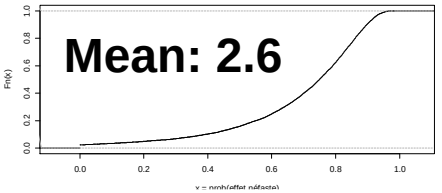
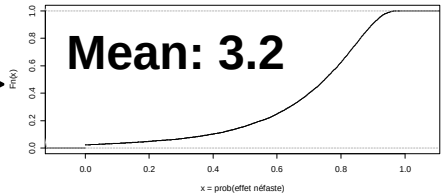
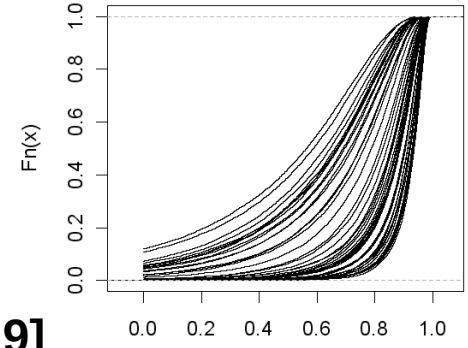




***N_u* times…**

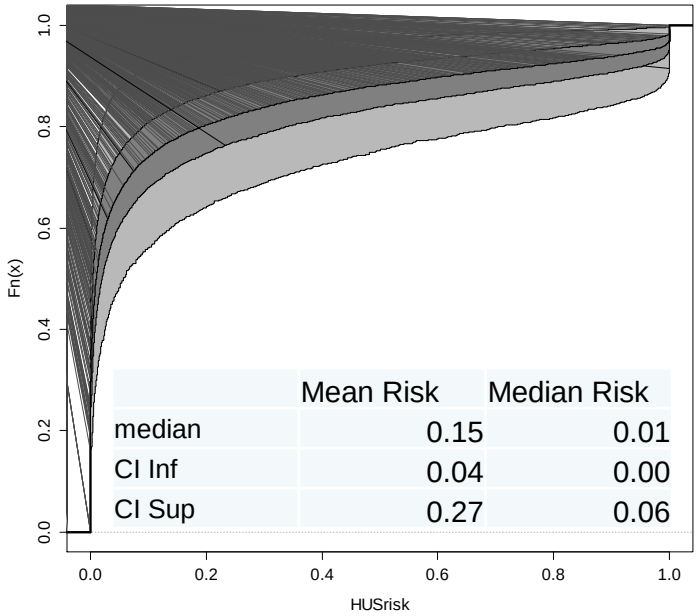


***N_u* Means: median 3.3, 2.5 and 97.5th percentiles [.2, 6.9]**

In practice

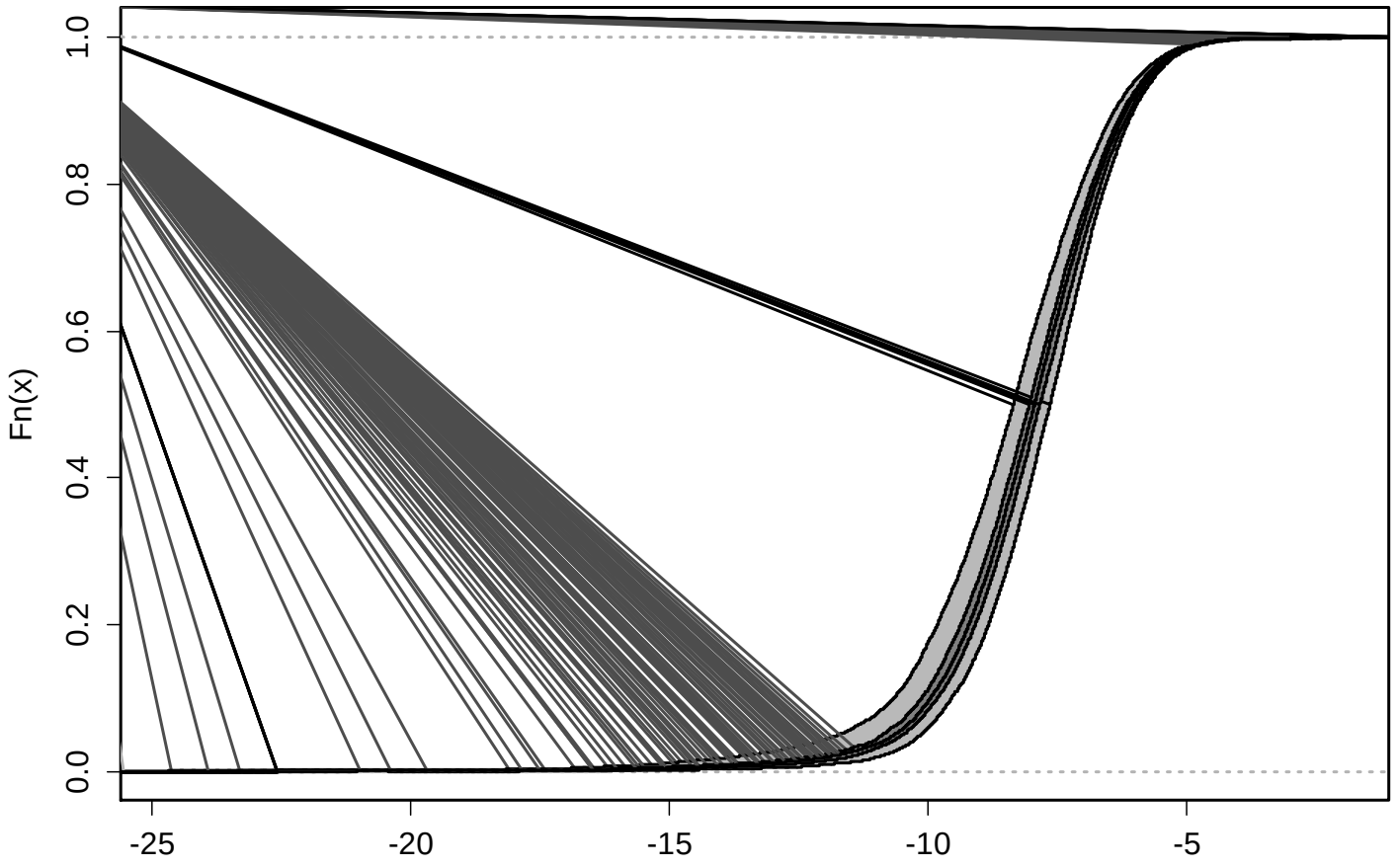
- Separation of uncertainty and variability is not explicitly considered in most MC software used in QMRA
- Can be done in classical MC software using large matrices or use other tools
- 2D-MC simulation to be implemented in FDA-iRISK® 3.0

- R package *mc2d* "Ease the development of MC and 2D-MC in R"
  - (you specify if the distributions represent Uncertainty or Variability, then *mc2d* do the math for you)



Variability cumulative distribution plots of the output of an *E. coli* model in ground beef model

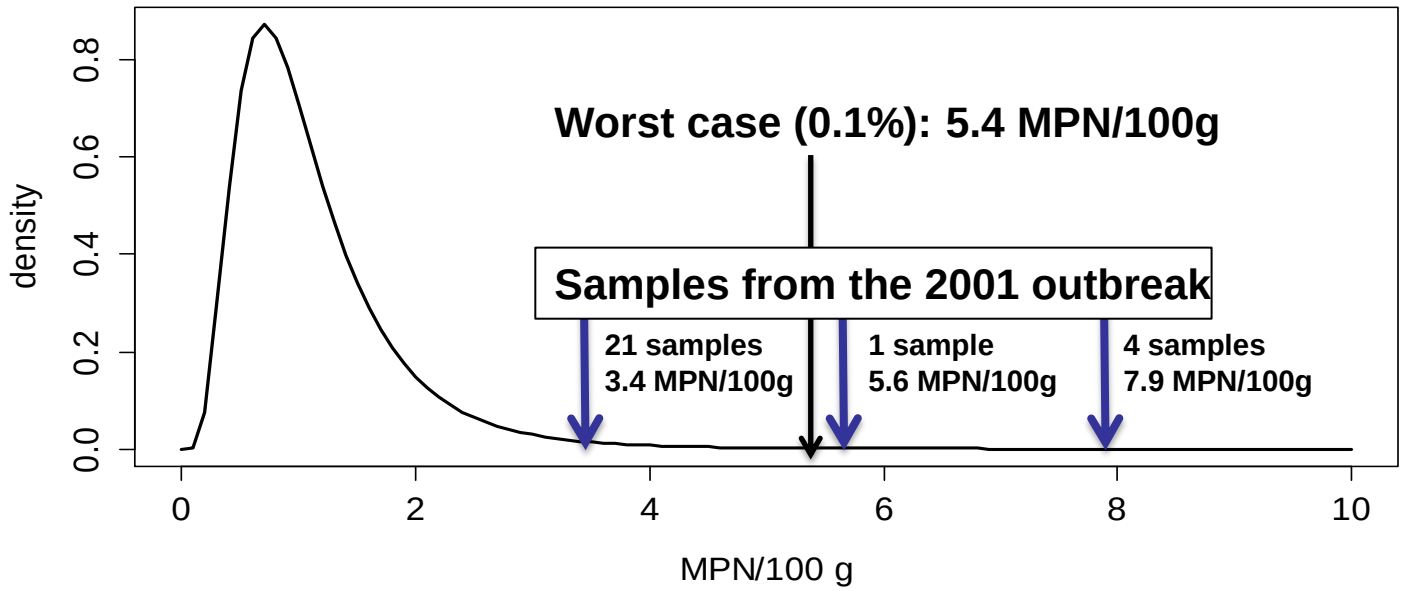
Results



Warning

- In this talk, we considered only data uncertainty within a given model
- Need to consider other sources of uncertainty
  - Scenario uncertainty
  - Model uncertainty
- May be much more important than the data uncertainty

Salmonella in almonds



- **The "outbreak situation" is not simply an extreme of the "usual situation".**
- **Needed to consider separately "usual situation" and "outbreak situation", as was done in this paper**

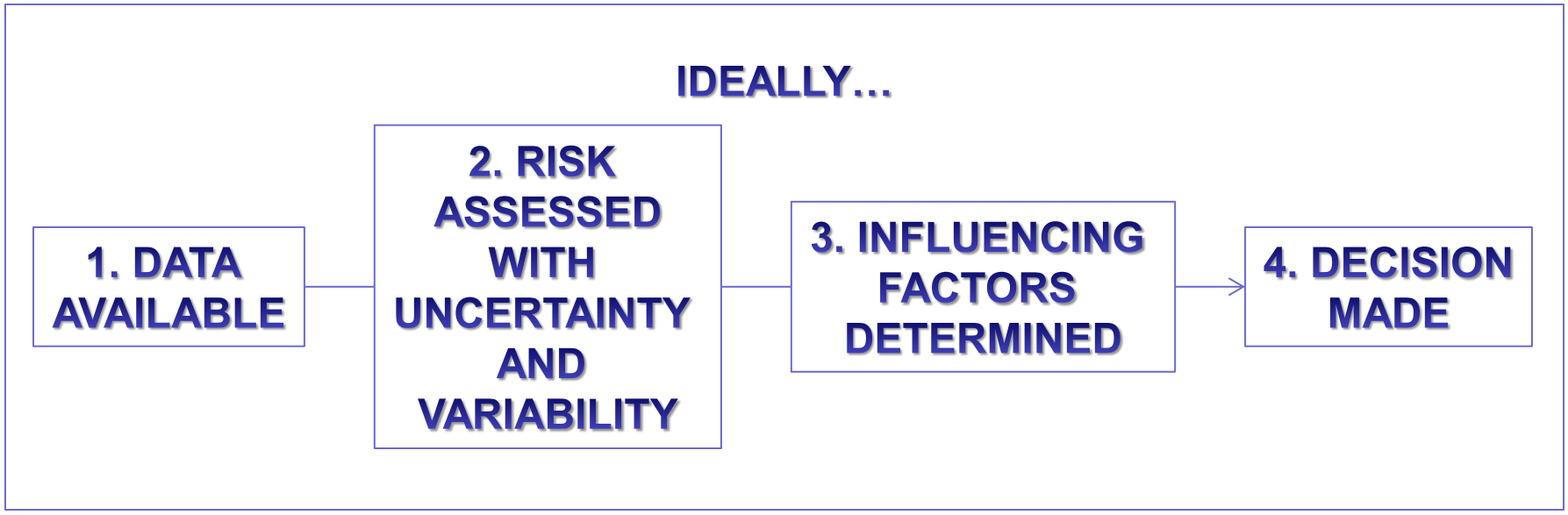
Lambertini, E., M. D. Danyluk, D. W. Schaffner, C. K. Winter, and L. J. Harris. 2012. Risk of salmonellosis from consumption of almonds in the North American market. *Food Research International*. 45:1166-1174.

Conclusions

- Don’t mix variability and uncertainty
  - Easiest way: consider variability only and test uncertainty for some major parameters
  - More complex methods available
  - Need carefully designed data collection for risk assessment purpose to consider proper variability
- Don’t forget Scenario and Model uncertainty
  - Need to model “exceptional events” that are not the extreme of the usual distribution
    - May lead your whole risk
    - Frequency? Magnitude?

How can we use the modeled variability and uncertainty for decision making?

Risk Management



SITUATION RARELY IDEAL, ASSUMPTIONS HAVE TO BE MADE

How to make a decision?

Log reduction treatment (log_{10})	Estimated mean number of cases		
	Estimate	CI 95%	
0	100,000	10,000	1,000,000
1	10,000	1,000	100,000
2	1,000	100	10,000
3	100	10	1000
4	10	1	100
5	1	<1	10
6	<1	<1	<1

Overall challenges in considering uncertainty and variability in risk analysis

- Risk assessors
  - Complicated process/ Not well understood
  - Feasibility
  - Lack of “easy-to-use” tools
- Risk managers
  - More difficult to handle
  - The uncertainty may be considered as “too large”
  - How to “draw a line”?
- Risk communication
  - More difficult to communicate
    - “So you are not certain?”

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